CLAIMS

1. A method for characterizing fluorescent molecules or other particles in samples comprising the steps of:

a) monitoring fluctuating intensity of fluorescence emitted by the molecules or other particles in at least one measurement volume of a non-uniform spatial brightness profile by measuring numbers of photon counts in primary time intervals by a single or more photon detectors,

b) determining at least one distribution of numbers of photon counts, $\hat{P}(\mathbf{n})$, from the measured numbers of photon counts,

c) determining physical quantities characteristic to said particles by fitting the experimentally determined distribution of numbers of photon counts $\hat{P}(\mathbf{n})$,

characterised in that the fitting procedure involves calculation of a theoretical distribution function of the number of photon counts $P(\mathbf{n})$ through its generating function, defined as $G(\vec{\xi}) = \sum_{n} \vec{\xi}^{\mathbf{n}} P(\mathbf{n})$.

2. A method according to claim 1 wherein the primary time intervals are consecutive intervals of equal width.

Sub A1

3. A method according to claim 1 or 2 wherein in step b) numbers of photon counts $\{\mathbf{n}_i\}$ subject to determination of a distribution $\hat{P}(\mathbf{n})$ are derived from numbers of photon counts in primary time intervals $\{\mathbf{N}_j\}$ by summing up numbers of photon counts from primary time intervals according to a predetermined rule.

4. A method according to claim 3 wherein in step b) numbers of photon counts $\{\mathbf{n}_i\}$ subject to determination of a distribution $\hat{P}(\mathbf{n})$ are calculated from the numbers of photon counts in primary time intervals $\{\mathbf{N}_j\}$ according to the rule $\mathbf{n}_i = \sum_{k=1}^{M} \mathbf{N}_{Mi+k}$, where M is an integer number expressing how many times the

time interval in which $\{\mathbf{n}_i\}$ is determined is longer than the primary time interval.

5. A method according to claim 3 wherein in step b) numbers of photon counts $\{\mathbf{n}_i\}$ are calculated from predetermined primary time intervals according to a rule in which primary time intervals are separated by a time delay.

6. A method according to claim 5 wherein in step b) numbers of photon counts $\{\mathbf{n}_i\}$ subject to determination of a distribution $\hat{P}(\mathbf{n})$ are calculated from the numbers of photon counts in primary time intervals $\{\mathbf{N}_j\}$ according to the rule $\mathbf{n}_i = \sum_{k=1}^{M}(\mathbf{N}_{Mi+k} + \mathbf{N}_{M(i+L)+k})$, where M and L are positive integer numbers.

Sub A2

7. A method according to one of the claims 3 to 6 wherein in step b) a set of distributions $\hat{P}(\mathbf{n})$ is determined according to a set of different rules, said set of distributions being fitted jointly in step c).

8. A method according to claims 6 and 7 wherein in step c) a set of distributions with different values of M and/or L are fitted jointly.

9. A method according to one of the claims 1 to 8 wherein at least one of the physical quantities of step c) is concentration of particles.

10. A method according to one of the claims 1 to 9 wherein at least one of the physical quantities of step c) is specific brightness of particles.

11. A method according to one of the claims 1 to 10, wherein at least one of the physical quantities of step c) is diffusion coefficient.

12. A method according to one of the claims 1 to 11 wherein the generating function is calculated using the expression $G(\xi) = \exp[\int dq\, c(q) \int d^3\mathbf{r}(e^{(\xi-1)qTB(\mathbf{r})} - 1)]$, where $c(q)$ is the density of particles with specific brightness q, T is the length of the counting interval, and $B(\mathbf{r})$ is the spatial brightness profile as a function of coordinates.

13. A method according to one of the claims 1 to 12 wherein the argument of the generating function is selected in the form $\xi = e^{-i\varphi}$ and a fast Fourier transform algorithm is used in calculation of the theoretical distribution of the number of photon counts out of its generating function.

14. A method according to one of the claims 1 to 13 wherein in step c) when calculating the theoretical distribution $P(\mathbf{n})$, the spatial brightness profile is modelled by a mathematical relationship between volume and spatial brightness.

15. A method according to claim 14 wherein in step c) when calculating the theoretical distribution $P(\mathbf{n})$, the spatial brightness profile is modelled by the following expression: $\frac{dV}{dx} = A_0 x(1 + a_1 x + a_2 x^2)$, where dV denotes a volume element, x denotes logarithm of the relative spatial brightness, A_0 is a constant selecting the unit of volume, and a_1 and a_2 are empirically estimated parameters.

16. A method according to claim 14 wherein in step c) when calculating the theoretical distribution $P(\mathbf{n})$, the spatial brightness profile is modelled by the following expression: $\frac{dV}{dx} = A_0 x^{a_3}(1 + a_1 x + a_2 x^2)$, where dV denotes a volume element, x denotes logarithm of the relative spatial brightness, A_0 is a constant selecting the unit of volume, and a_1, a_2 and a_3 are empirically estimated parameters.

Sub A3

17. A method according to any of the claims 1 to 16 wherein in step a) a confocal optical device is used for monitoring the intensity of fluorescence.

18. A method according to any of the claims 1 to 17 wherein said fluorescent molecules or other particles are characterized applying an homogeneous fluorescence assay.

19. A method according to any of the claims 1 to 18 for use in diagnostics, high throughput drug screening, optimization of properties of molecules and identification of specific cell or suspendable carrier populations.

20. Use of a confocal apparatus for performing the method according to any of the claims 1 to 19 comprising:
a radiation source (12) for providing excitation radiation (14),
an objective (22) for focussing the excitation radiation (14) into a measurement volume (26),
a detector (42) for detecting emission radiation (30) that stems from the measurement volume (26), and
an opaque means (44) positioned in the pathway (32) of the emission radiation (30) or excitation radiation (14) for erasing the central part of the emission radiation (30) or excitation radiation (14).